Perfused liver health assessment employing Raman spectral analysis: A review

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Introduction

Challenges with diagnostic instrumentation during organ transplant pose potential life-death consequences and hence the need for a label-free and predictive monitoring system. In this publication review, a novel Raman-based diagnostic tool is proposed for use in monitoring the overall health and degradation over time of ex vivo perfusion of porcine livers. The Raman-based approach involved Raman spectral analyses of perfusate fluids from pigs. A combination of multivariate statistical analyses (MVA), including principal component analysis (PCA) and linear discriminant analysis (LDA), was employed on the spectral data to infer degradation profiles for perfusates collected over different conditions of pressure, temperature and over time. This study shows that the technique was sensitive to detect transitions between healthy livers and the gradual transition to unhealthy or degenerate state over time. The technique, when optimized, has potential applications in perfusion and diagnostic instrumentation for on-the-go analysis during organ transit and in operating rooms to help with quick identification pre-transplant of organ health.

What is Raman Spectroscopy?

Discovered by Dr. C.V. Raman in 1928, Raman Spectroscopy is a commonly used technique. This non-destructive form of chemical analysis is used to observe the rotation, vibrational, and low-frequency modes in a system. Known as a 'Fingerprinting' method, this technique is inelastic scattering of 1 million photons off a molecule rather than absorption. Raman Scatter, the two photon process, describes occurrence when the scattering light and incident light do not possess the same frequency as a result of the difference between oscillation of light and molecular vibration.

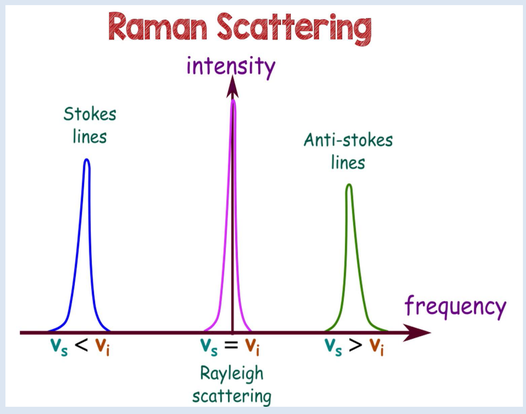

Figure 1 . Distinction between Rayleigh and Raman scattering

Raman Spectroscopy is used to determine the stress strain, measure of crystallinity, and material identification and verification (a.k.a. Molecular Fingerprint), chemical structure, phase and morphology, and molecular interactions

Instrument setup and optimization

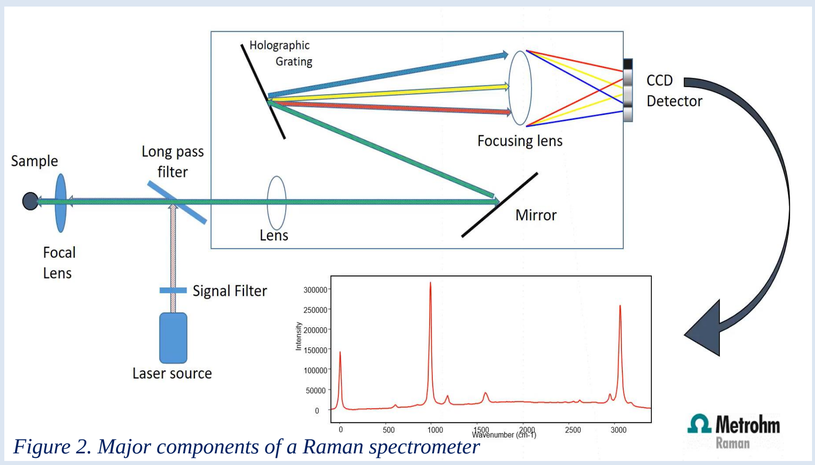

Figure 2. Major components of a Raman spectrometer

Three major Components

1. Laser - Patented technology. Narrow bandwidth and high stability which enables the user to see sharp, clear peaks in the results with less deviation of the measured spectrum
2. Probe fiber optic or Microscope - guides the laser into a bandpass filter then the probe's shaft. It focuses the laser and collects the back scatter, passing it into edge filter, guiding to the spectrometer. (sampling apparatus)
3. Spectrometer

Key Insights

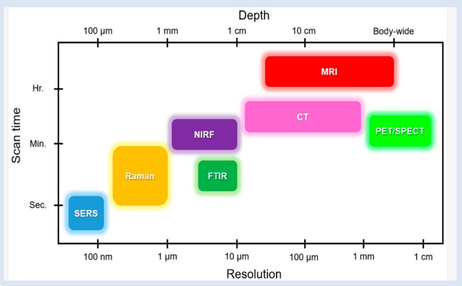

Figure 3. Comparison of Raman spectral resolution against other techniques

Knowing the shifts and relative intensities of the Raman bands (of the material) make identification of the sample possible. Bands may vary in broadness or narrowness, intensity, and/or may shift, revealing information about stresses within the sample, amount present, and crystallinity. The spectral variations determine the homogeneity of the material and composition.

Results

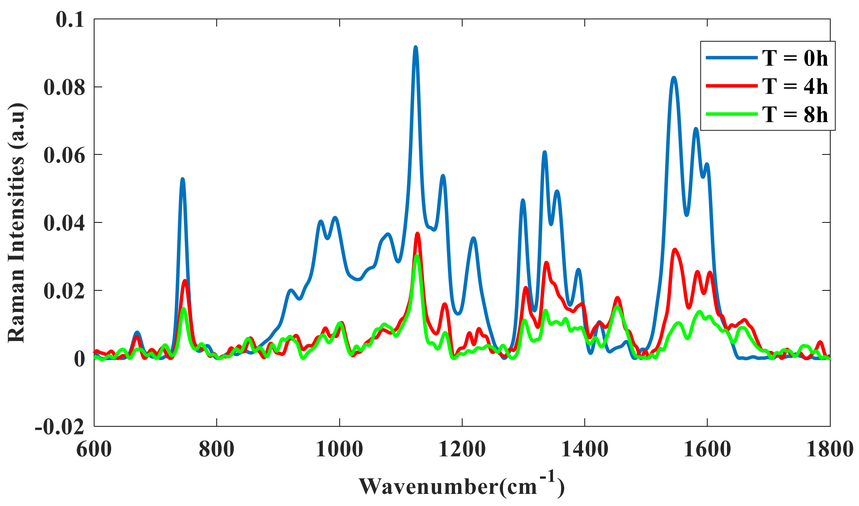

Figure 4. (a) Raman spectra of perfused porcine liver at the following time points: 0 h before perfusion begins (blue), 4 h of perfusion (red), and 8 h of perfusion (green)

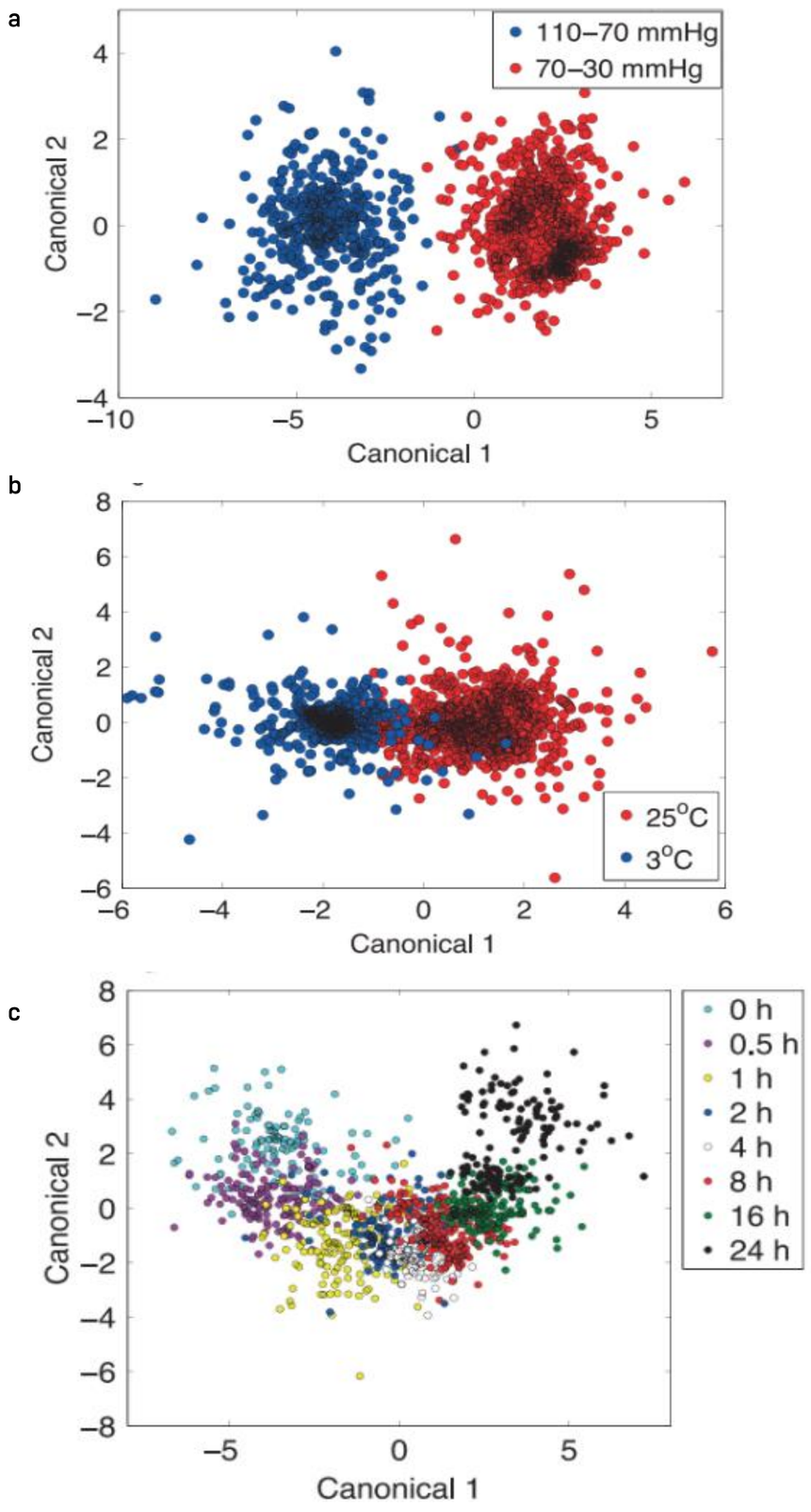

Figure 5 (a) all data points for livers perfused under conditions A, B, and C discriminated by pressure, (b) all data points for livers perfused under conditions A, B, and C discriminated by temperature, and (c) all data points for livers perfused under conditions A, B, and C discriminated by time.

Data Collection and Analysis

Experimental conditions: 70-30 mmHg at 3ºC, 110-70 mmHg at 25ºC, and 70-30 mmHg at 25ºC. Multivariate statistical analysis applied. Perfusate conditions and times held a significant impact on the Raman spectra. Linear Discriminate Analysis was performed used to determine degradation. Perfusion pressure held larger impact on liver degradation in comparison to the temperature; however, this was not witnessed in the second set of livers

Experimental set-up

- 6 livers were harvested and flushed of whole blood with ice cold (4°C) modified Krebs–Henseleit solution, with added heparin anticoagulant, and stored on ice.
- After 2 hours, the livers were transported to the lab and active perfusion was initiated for 24 hours, consisting of a perfusate reservoir bag, cardiovascular emulation system pump, heat exchanger, pressure regulator, atrial line, perfused liver, ventricular line, and recycle stream.
- 10 µL of perfusate fluid was air dried on an aluminum surface at room temperature and analyzed by Raman Spectroscopy.
- Extraction of liver perfusate fatty acids.
- Protein in perfusate was measured (triplicate per sample) and absorbance was analyzed.
- Statistical analyses were performed to: identify correlations between Raman spectral bands and measured fatty acid and protein data; identify sample outliers; and separate and cluster Raman spectra according to liver treatment parameters.

Conclusion

Clear distinctions based on selected conditions were detected within the 24 hours. A larger sample size would be required to make conclusive statements regarding the effects of perfusion conditions. The goal of the study was to develop and demonstrate Raman spectroscopy statistical analysis as a potential diagnostic tool. It is ideal to use the multivariate statistical analysis method for the closed system. Despite the advantages, challenges remain for linking changes in perfusate fluid to organ health. As these techniques continue to improve, clinicians will be able to use this real-time technique for making critical decisions when determining viability of an organ.

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References

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